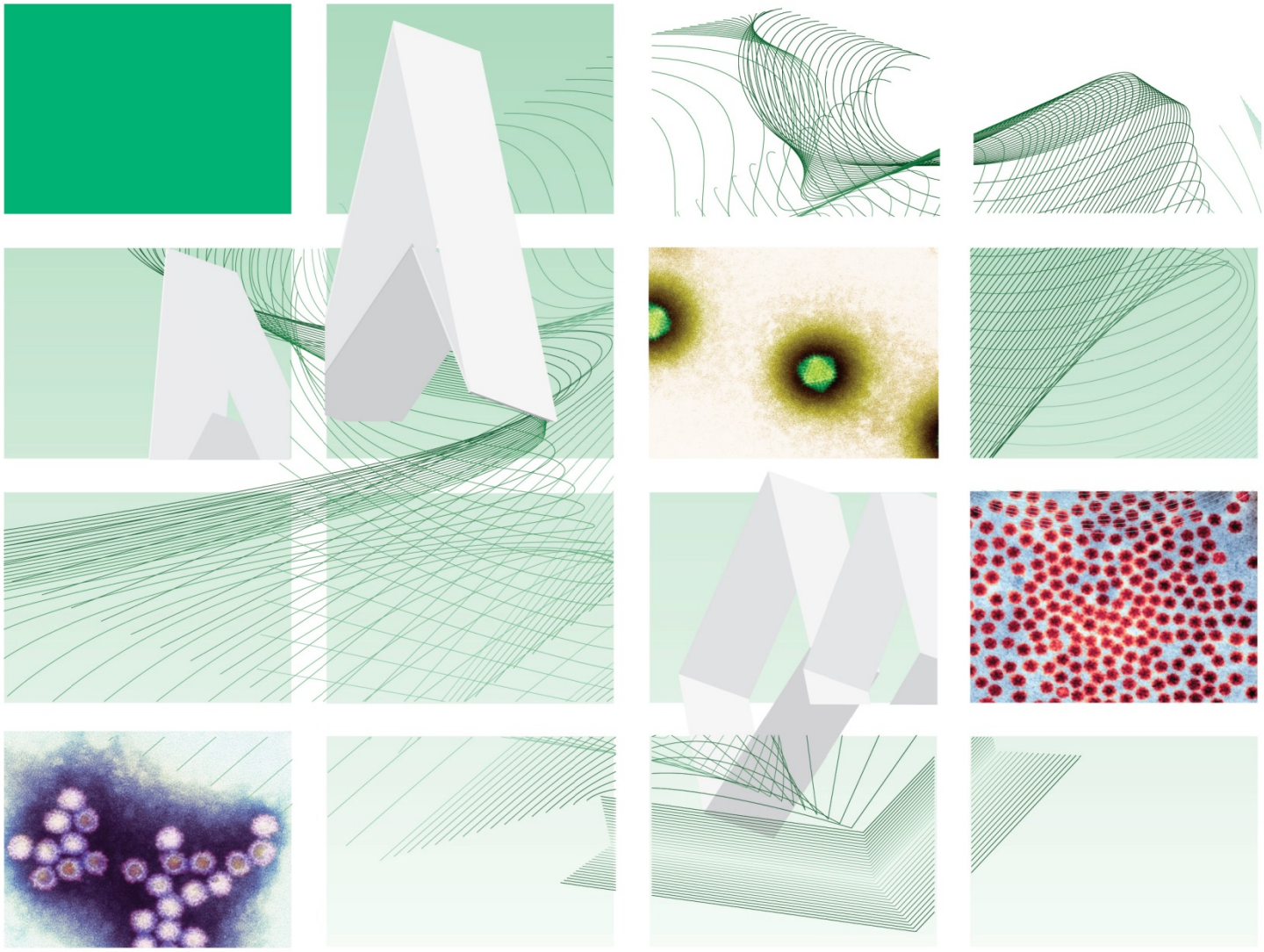
Public Health England

NHS

UK Standards for Microbiology Investigations

Screening for hepatitis C infection



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"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Issued by the Standards Unit, Microbiology Services, PHE

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Contents

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	9/01.08.17
Issue number discarded	6.2
Insert issue number	7
Anticipated next review date*	31.07.20
Section(s) involved	**Amendment**
Whole document.	Introduction updated to include background information on Hepatitis C virus. Document updated to include sections: Technical Limitations, Safety Considerations, Public Health Management and Report Comments. References updated.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

Suggested citation for this document

Public Health England. (2017). Screening for hepatitis C infection. UK Standards for Microbiology Investigations. V 5 Issue 7. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

EDTA whole blood, EDTA plasma, serum

This UK SMI covers the screening of blood, plasma and serum samples for hepatitis C (HCV) using HCV antibody Enzyme Immunoassays (EIA) screening tests as well as confirmation using Nucleic Acid Amplification Tests (NAAT), Immunoblots and HCV core antigen EIA assays. Dried Blood Spot (DBS) samples are increasingly employed in hard-to access populations, as a public health tool in prison services and in people who inject drugs (PWID). DBS samples can be used to collect whole blood specimens which are tested using standard CE-marked Anti-HCV EIA and NAAT assays after verification and validation by accredited testing laboratories[1].

Reflex testing is recommended. HCV RNA NAAT performed on the same sample as the original screening assay streamlines the HCV care pathway and avoids losing the 20-30% of patients with positive screening tests for whom a second sample is never received[2]. Reflex NAAT testing decreases the turnaround time for referral, benefits patient care and increases cost effectiveness[3,4].

Further studies are currently required to determine the relative sensitivities and specificities of combined antigen/antibody assays[5,6].

Commercial NAATs may not be validated for all sample types listed above. Manufacturers' recommendations should be followed and all kits should be validated and verified locally prior to use.

For the investigation and management of occupational exposure, refer to PHE and HSE guidelines[7,8].

For blood-borne virus (BBV) testing following dialysis away from base (DAFB), refer to DH guidelines[9].

Refer to SaBTO guidance for information regarding screening for HCV in the case of blood or organ donation and transplantation[10].

This UK SMI should be used in conjunction with other UK SMIs.

Abbreviations

Abbreviation	Definition
HCV	hepatitis C virus (complete infectious virion)
HCV core Ag	hepatitis C core antigen
Anti-HCV	Antibody to HCV
BBV	Blood-borne viruses

Definitions

For all antigen, antibody and NAAT testing, the following definitions apply:

During testing process

Reactive – Initial internal-stage positive result pending confirmation.

Not reactive – Initial internal-stage negative result.

Equivocal – Result is not clearly positive or negative. Further testing is required.

The term 'equivocal' may be different for various platforms for example 'indeterminate'.

Inhibitory – The term 'inhibitory' may be different for various platforms for example 'invalid'.

Reporting stage

These terms are used for final or preliminary reports.

Detected – Report-stage confirmed reactive result.

Not detected – Report-stage not reactive result.

Indeterminate – Reactive result that cannot be confirmed.

Inhibitory – The term 'inhibitory' may be different for various platforms for example 'invalid'.

Introduction

Hepatitis C virus (HCV) is a blood-borne virus of the *Flaviviridae* family and a member of the Hepacivirus genus[11-13]. It is a single stranded, positive sense enveloped RNA virus with a genome of approximately 9600 bases[11]. According to the World Health Organization (WHO), more than 185 million people worldwide are infected with hepatitis C virus and, of these, 700,000 die every year[14]. In the UK, 214,000 people are estimated to be living with chronic HCV infection[1]. There are also 10 million people worldwide estimated to be co-infected with HCV and HIV, possibly associated with high-risk traumatic sexual practices and drug use in men who have sex with men (MSM)[15]. As effective medical treatments can dramatically improve the prognosis, early detection programs may prevent the development of serious chronic conditions, improve health, prevent ongoing transmission and therefore save resources.

WHO has adopted a Global Health Sector Strategy (GHSS) target that HCV-related mortality should be reduced by 10% in 2020 and 65% by 2030. The UK is working towards elimination of HCV using the increasingly available new direct-acting antivirals (DAA)[1,16].

HCV infects the liver and transmission of HCV occurs mainly via contact with blood or blood-derived products. Thus, the main risk factors for acquiring infection are the use of (or accidental injury involving) contaminated blood or blood products, contaminated needles or syringes, and inadequately sterilised medical/dental instruments[17]. The most frequent modes of transmission vary between the developed and developing world[17]. In the developed world, since the introduction of screening of blood and blood-derived products, the primary mode of transmission has been through injecting recreational drug use[18]. In the UK, HCV remains common in people who inject drugs (PWID), with 10% and 70% antibody prevalence for those injecting for 2 years and 15 years respectively[19]. In developing countries, where sterile medical supplies are short or non-existent, routine injections and medical/dental procedures may confer a high cumulative lifetime risk of acquiring HCV[17]. More infrequent transmission sources are body modification (tattooing and body piercing), occupational, perinatal and sexual exposures[20]. Prevalence of HCV infection in the UK is higher in people who received

blood transfusions, tissue/organ transplants or blood products before the introduction of sensitive screening tests in 1992[21].

Acute HCV infection is often asymptomatic (85-90% of cases) and therefore is rarely diagnosed[18,22,23]. Symptoms, if they occur, may include jaundice, nausea and malaise; fulminant hepatitis is extremely rare[22]. Spontaneous viral clearance is very rare beyond 4 to 6 months of infection, thus HCV RNA detectable for longer than 6 months is considered chronic infection[24]. Sequelae of chronic HCV infection, including end-stage cirrhosis and hepatocellular carcinoma (HCC), may develop less than 20 years to greater than 30 years after the initial infection[22]. Co-infection with HIV accelerates disease progression as HCV behaves as an opportunistic infection[17]. Co-infection with hepatitis B virus results in synergistic progression of both infections[17]. Increased alcohol intake also accelerates the progression of chronic HCV towards end-stage cirrhosis and HCC[17]. Transplantation is often necessary following HCV related liver failure, but progression towards cirrhosis is reported in over 25% of patients within 5 years of transplantation[25].

HCV reinfection is defined by the reappearance of HCV RNA at least 6 months after a sustained viral clearance (SVR) and the demonstration that infection is due to a different HCV genotype or strain (determined by phylogenetic analysis if the genotype is the same)[26].

HCV screening in people who may be at increased risk of infection has been strongly recommended by WHO guidelines as well as guidelines from UK bodies (such as Public Health England, British HIV Association and the British Association of Sexual Health and HIV) to address the need to improve rates of earlier diagnosis[1,14,26-28].

The relationship between neutralising antibodies and the control of HCV viraemia is complex due to the enormous genetic variability of the virus (much greater than the variability seen with HIV), particularly in the E2 envelope glycoprotein region where many antibodies are targeted[29]. This variability also leads to HCV existing as a “quasispecies” or group of closely-related variant viruses, in each infected host[11]. The window between detection of HCV RNA and antibodies to HCV can be months, with an average of 60 days[30].

There are six genotypes of HCV (1-6) with multiple subtypes, although a new genotype has been proposed as genotype 7[22,31,32]. Subtypes are designated alphabetically in order of their discovery. HCV genotypes 1, 2, and 3 are distributed worldwide, with geographic variation in their relative prevalence. HCV genotype 4 is prevalent in North Africa, Central Africa and the Middle East, while genotypes 5 and 6 seem to be confined to South Africa and Hong Kong, respectively. The most common genotypes occurring in the UK are 1 and 3[33,34].

Laboratory diagnosis

Routine laboratory diagnosis of established infection is based upon the detection of antibodies for the virus using serological methods, followed by the detection of the virus using nucleic acid amplification testing or antigen testing to confirm viraemia[11,21,28,35-37]. Antibodies to HCV are detected using Enzyme Linked Immunosorbent Assays (ELISA), Enzyme Immunoassays (EIA), Chemiluminescent Immunoassays (CLIA) or Immunoblots[38]. Assays have been developed to detect antibodies to an increasing range of viral proteins, from second generation (core proteins and non-structural proteins 3 and 4), third generation (also with non-structural protein 5) and now fourth generation (also with HCV capsid antigen)[22,37].

HCV RNA NAAT is preferable to an HCV core antigen test for confirmation. HCV core antigen assays are less sensitive than NAAT, with a limit of detection equivalent to 500-3000 IU/mL HCV RNA, depending on the HCV genotype[39]. The HCV core antigen test is reported to have a 100% specificity and 96 - 97% sensitivity when compared with HCV RNA NAAT[40,41]. Laboratories should be aware that NAAT should be considered for patients who are HCV core antigen negative.

Some CE-marked assays require serum or plasma for NAAT to be separated within 6hrs of phlebotomy, which is difficult to achieve for some laboratories. However, a (qualitatively) positive HCV antigen or NAAT result, which indicates active infection, is often sufficient to identify appropriate treatment regimens. In addition, it may be challenging to obtain an additional repeat sample to confirm active infection in those who are hard to recall or difficult to bleed.

The presence of RNA and antibodies to HCV do not confirm whether a current infection is acute or chronic[30]. However, antibodies can take a long time to develop and are therefore only detectable in 50-70% of symptomatic acute infections[23].

HCV RNA in blood is a good marker of replicating virus and it may be detected as early as 1-3 weeks after initial infection[30]. Molecular methods can also be used to distinguish between the different HCV genotypes and subtypes, to guide treatment selection and differentiate relapse from reinfection[22].

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers[42,43]

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Safety considerations

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[44].

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

1 Specimen transport, storage and retention[42,43]

1.1 Optimal transport and storage conditions

Specimens should be transported and processed as soon as possible[45].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[45].

Note: Specimens for NAAT can be stored long-term at -20° or -70°C to minimise RNA loss[46].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[47].

Public health management

Hepatitis C is usually asymptomatic for many years after infection, therefore numerous individuals remain undiagnosed.

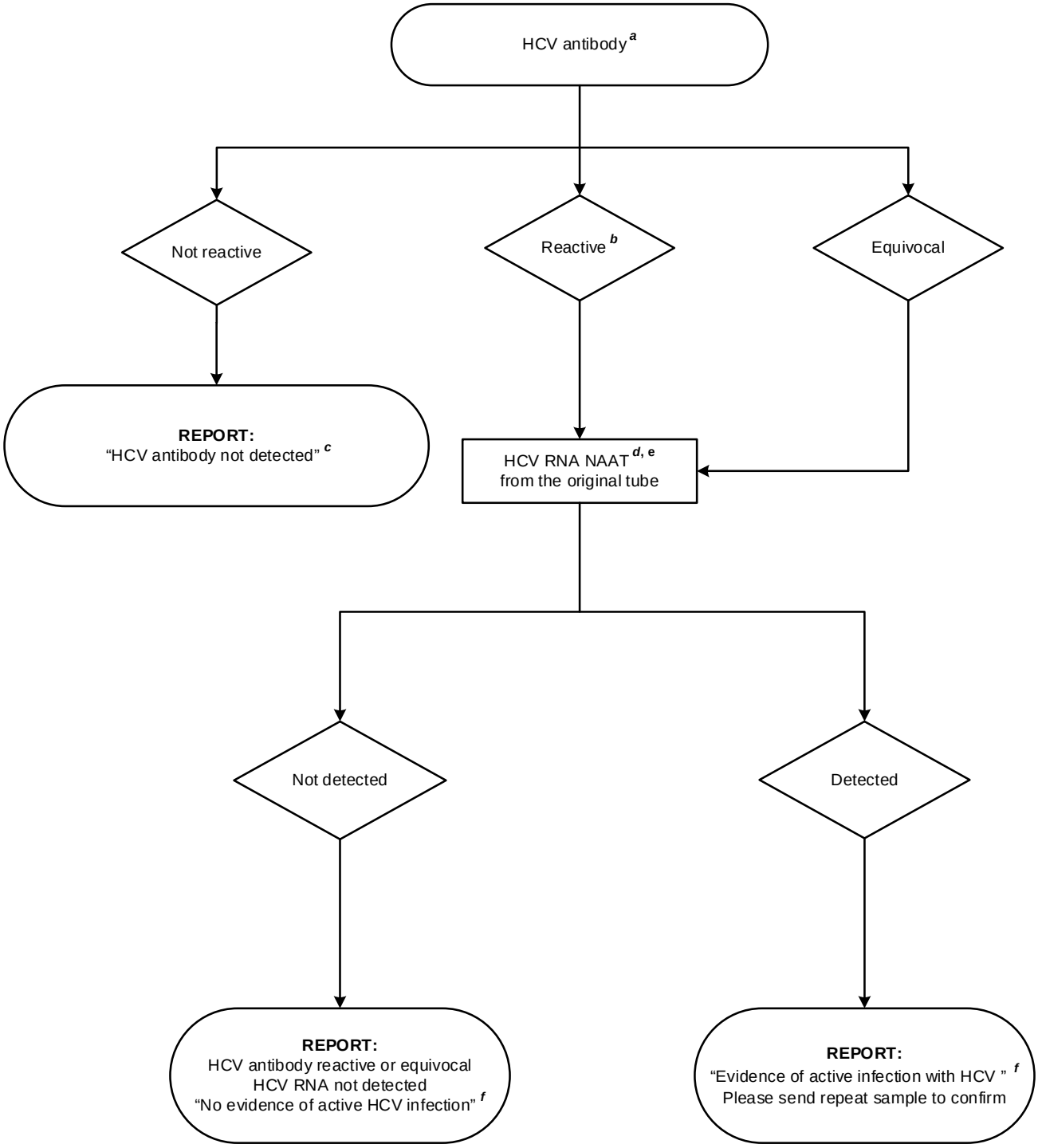
For information regarding notification to PHE (or equivalent in the devolved administrations) refer to page 19.

For further information on public health management refer to PHE guidance: https://www.gov.uk/government/collections/hepatitis-c-guidance-data-and-analysis and www.gov.uk/government/publications/hepatitis-b-and-c-local-surveillance-standards.

In addition to reporting new positive diagnosis to PHE Health Protection Teams, participating laboratories should also report into sentinel surveillance programmes for HCV.

In the UK, guidance for hepatitis C infected health care workers (HCW) is available[48]. See link: https://www.gov.uk/guidance/bloodborne-viruses-in-healthcare-workers-report-exposures-and-reduce-risks

Investigation of hepatitis C infection by HCV antibody testing confirmed by HCV RNA NAAT[35,49,50]



Footnotes for algorithm showing HCV antibody testing confirmed by HCV RNA NAAT

a) For immunocompromised patients, who may have a delayed antibody response, cases strongly suspected to be due to acute HCV or where re-infection or re-activation is suspected, alternative screening with NAAT or HCV core antigen may be indicated. It should be noted that screening using HCV core antigen or NAAT testing is increasingly used for renal dialysis units[9,51,52].

b) Issue an interim report if confirmation will be delayed and the result may have immediate significance for patient management; suggested wording ‘Initial HCV antibody reactive. Awaiting confirmation’.

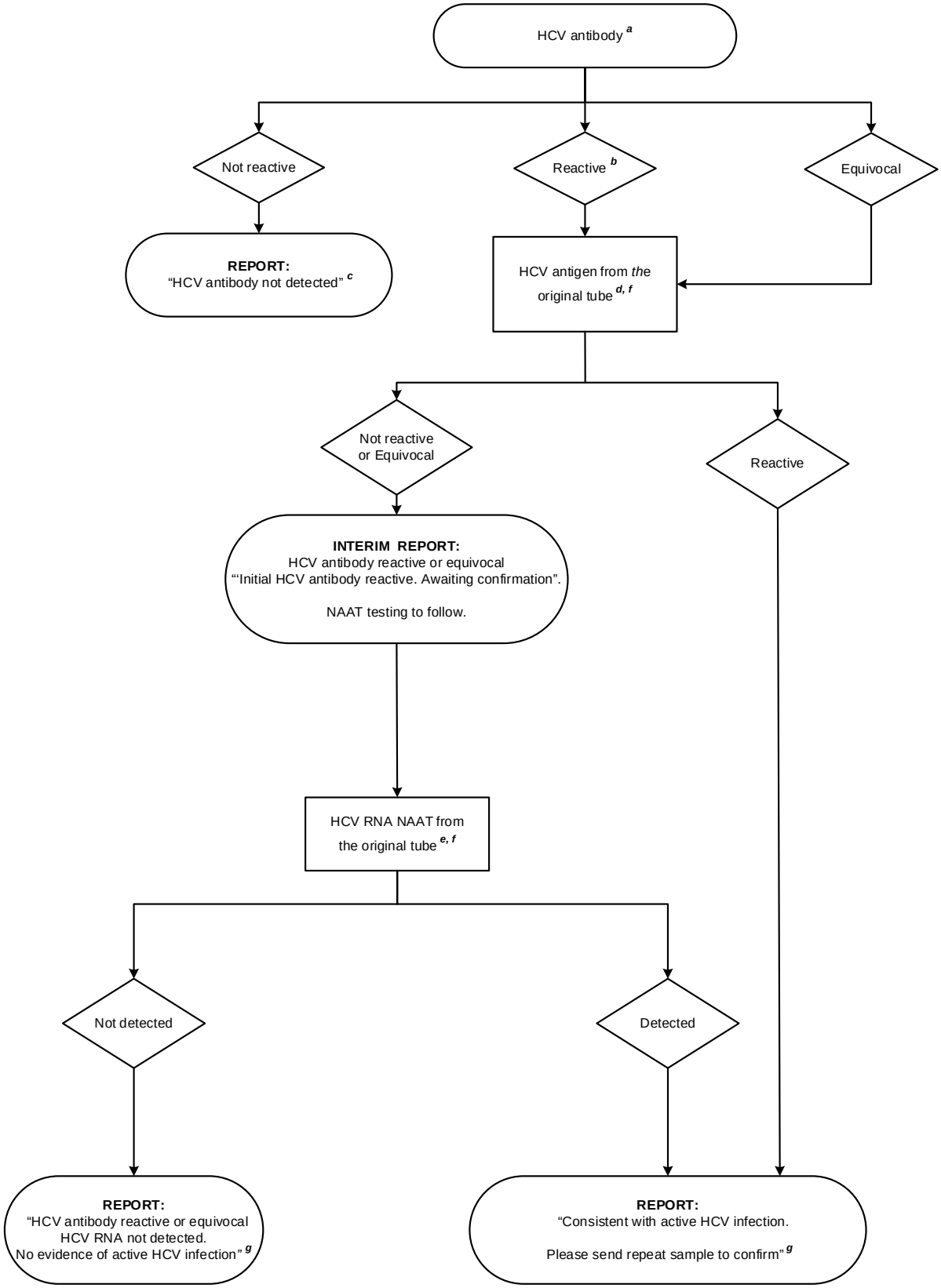
c) If occupational exposure risk, request a repeat sample at an appropriate interval, usually a sample at 6 and 12 weeks for NAAT and at 12 and 24 week for antibody test[7,48]. Most people will be NAAT positive by 4 weeks post exposure and antibody positive by 12 weeks post exposure. Follow local guidelines when dealing with occupational health.

d) Qualitative or quantitative NAAT can be used to demonstrate the presence of HCV RNA. High levels of sensitivity can only be achieved with recommended sample volumes; if a suboptimal volume is used (perhaps diluted) this must be reported and a repeat sample requested. All assays should include appropriate controls, including an inhibition control, and manufacturers’ recommendations should be followed. Where sample preparation protocols or assays are being utilised “off label” local validation should be performed prior to use.

e) Request a second specimen where there is insufficient sample volume to complete testing. Specimen type, that is, EDTA or serum for repeat sample as per local guidance.

f) Refer to report table for interpretation of results.

Investigation of hepatitis C infection by HCV antibody testing confirmed by HCV core antigen[35]



Footnotes for algorithm showing HCV antibody testing confirmed by HCV core antigen

a) For immunocompromised patients, who may have a delayed antibody response or cases strongly suspected to be due to acute HCV or where re-infection or re-activation is suspected, immediate screening with NAAT or HCV core antigen may be indicated. It should be noted that screening using HCV core antigen or NAAT testing is increasingly used for renal dialysis units[9,51,52].

b) Report at this stage as an interim report if confirmation will be delayed and the result may have immediate significance for patient management; suggested wording 'Initial HCV antibody reactive. Awaiting confirmation'.

c) If occupational exposure risk, request a repeat sample at an appropriate interval, usually a sample at 6 and 12 weeks for NAAT and at 12 and 24 week for antibody test[7,48]. Most people will be NAAT positive by 4 weeks post exposure and antibody positive by 12 weeks post exposure. Follow local guidelines when dealing with occupational health.

d) HCV core antigen is a surrogate marker of HCV replication and can be used to diagnose active infection when HCV RNA NAAT is not available or not affordable[26]. HCV core antigen assays are less sensitive than HCV RNA NAATs.

e) Qualitative or quantitative NAAT can be used to demonstrate the presence of HCV RNA. High levels of sensitivity can only be achieved with recommended sample volumes; if a suboptimal volume is used (perhaps diluted) this must be reported and a repeat sample requested. All assays should include appropriate controls, including an inhibition control, and manufacturers' recommendations should be followed. Where sample preparation protocols or assays are being utilised "off label"' local validation should be performed prior to use.

f) Request a second specimen where there is insufficient sample volume to complete testing. Specimen type, that is, EDTA or serum for repeat sample as per local guidance.

g) Refer to report table for interpretation of results.

Report comments[50]

The final result should be able to distinguish active HCV infection from resolved infection using a combination of antibody, antigen and NAAT tests.

Following an initial (first sample) positive result it is best practice to request a repeat sample.

Investigation of hepatitis C infection by HCV antibody testing confirmed by NAAT					
	1st Assay	**2nd Assay**	**3rd Assay Optional**	**Interpretative comments**	**Notes**
	HCV Ab	**HCV NAAT**	**HCV Ab**		
1	Not reactive	Not tested	Not tested	HCV antibody not detected.	In the case of suspected acute hepatitis C or in immunocompromised patients, HCV RNA testing should be part of the initial evaluation.
2	Reactive or Equivocal	RNA Detected	Not tested	HCV antibody reactive. HCV RNA detected. Evidence of active HCV infection. Advise referral to an appropriate specialist for further assessment/treatment. Hepatitis A and B vaccine recommended if appropriate.	HCV antibody detected in the presence of HCV RNA allows one to infer with confidence that the HCV antibody reaction is a true positive. Please ensure hepatitis A and B status is known and vaccination given if needed. Consider requesting HCV genotyping and other BBV testing unless already performed. If initial antibody assay is equivocal and RNA detected, this may be recent infection. Consider review of clinical and results history, that is, can seroconversion to anti-HCV antibodies be documented.
3	Reactive or Equivocal	RNA not detected	Not tested	No evidence of active HCV infection.	EASL 2016 recommend that “Anti-HCV positive, HCV RNA negative individuals should be retested for HCV RNA 3 months later to confirm definitive clearance”[26].

Investigation of hepatitis C infection by HCV antibody testing confirmed by NAAT					
	1st Assay	**2nd Assay**	**3rd Assay Optional**	**Interpretative comments**	**Notes**
	HCV Ab	**HCV NAAT**	**HCV Ab**		
4	Reactive or Equivocal	RNA not detected	Reactive or Equivocal	No evidence of active HCV infection.	HCV antibody positive result may indicate past HCV infection. EASL 2016 recommend that "Anti-HCV positive, HCV RNA negative individuals should be retested for HCV RNA 3 months later to confirm definitive clearance"[26]. Suggest a repeat sample to confirm HCV antibody status. Please note that undetectable HCV RNA does not exclude current infection because viraemia may be intermittent. Suggest testing a follow-up blood for HCV NAAT to investigate possible fluctuating viraemia[49, 26].
5	Reactive or Equivocal	RNA not detected	Not reactive	No evidence of active HCV infection.	Consider reporting initial HCV Ab reactivity according to local policy.
6	Not reactive	RNA detected	Not tested	HCV RNA detected. Evidence of active HCV infection. Advise referral to an appropriate specialist for further assessment/treatment. Hepatitis A and B vaccine recommended if appropriate.	Indicates either acute HCV infection or possibly a chronic infection in an immunocompromised patient. Please ensure hepatitis A and B vaccination status is known and vaccination given if needed. Consider requesting HCV genotyping and other BBV testing unless already performed.

Investigation of hepatitis C infection by HCV antibody testing confirmed by antigen					
	1st Assay HCV Ab	**2nd Assay HCV Ag**	**3rd assay HCV NAAT**	**Interpretative comments**	**Notes**
7	Not reactive	Not tested	Not tested	HCV antibody not detected.	In the case of suspected acute hepatitis C or in immunocompromised patients, HCV RNA testing should be part of the initial evaluation. HCV core antigen assays are less sensitive than HCV RNA assays as a result core antigen becomes detectable in peripheral blood a few days after HCV RNA in patients with acute HCV [26].
8	Reactive or Equivocal	Reactive	Not tested	Consistent with active HCV infection. Advise referral to an appropriate specialist for further assessment/treatment. Hepatitis A and B vaccine recommended if appropriate.	Consider HCV RNA testing in addition to requesting HCV genotyping testing. Recommend other BBV testing unless already performed.
9	Reactive or Equivocal	Equivocal or Not reactive	Not detected	No evidence of active HCV infection.	Please send a repeat sample to confirm by NAAT. EASL 2016 recommend that "Anti-HCV positive, HCV RNA negative individuals should be retested for HCV RNA 3 months later to confirm definitive clearance"[26]. Recommend other BBV testing unless already performed.
10	Reactive or Equivocal	Equivocal	Detected	HCV RNA detected. Evidence of active HCV infection. Advise referral to an appropriate specialist for further	Consider HCV RNA testing in addition to requesting HCV genotyping testing. Recommend other BBV testing unless already performed.

Investigation of hepatitis C infection by HCV antibody testing confirmed by antigen					
	1st Assay HCV Ab	**2nd Assay HCV Ag**	**3rd assay HCV NAAT**	**Interpretative comments**	**Notes**
				assessment/treatment. Hepatitis A and B vaccine recommended if appropriate.	

Notification to PHE[53,54], or equivalent in the devolved administrations[55-58]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[55,56], Wales[57] and Northern Ireland[58].

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	**Quality of evidence**
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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2. McGibbon E, Bornschlegel K, Balter S. Half a diagnosis: gap in confirming infection among hepatitis C antibody-positive patients. The American journal of medicine 2013;126:718-22. **B, II**

3. Gale HB, Dufour DR, Qazi NN, Kan VL. Comparison of serial Hepatitis C virus detection in samples submitted through serology for reflex confirmation versus samples directly submitted for quantitation. Journal of clinical microbiology 2011;49:3036-9. **B, II**

4. Chapko MK, Dufour DR, Hatia RI, Drobeniuc J, Ward JW, Teo CG. Cost-effectiveness of strategies for testing current hepatitis C virus infection. Hepatology 2015;62:1396-404. **B, II**

5. Yang JF, Lin YY, Hsieh MH, Tsai CH, Liu SF, Yu ML et al. Performance characteristics of a combined hepatitis C virus core antigen and anti-hepatitis C virus antibody test in different patient groups. The Kaohsiung journal of medical sciences 2011;27:258-63. **B, II**

6. Yagci S, Padalko E. Comparison of monolisa HCV Ag/Ab ULTRA with two anti-HCV assays for the detection of HCV infection in hospital setting. Current microbiology 2012;64:148-51. **B, II**

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